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TI A strain of rhesus rhadinovirus (RRV 17577) related to Kaposi's sarcoma-associated herpesvirus (KSHV) encodes a functional homologue of cellular interleukin-6.
AU Kaleeba, Johnan A. R. (1); Bergquam, Eric P. (1); Wong, Scott W. (1)
CS (1) Division of Pathobiology and Immunology, Oregon Regional Primate Research Center, Beaverton, OR, 97006 USA
SO Journal of Medical Primatology, (Aug. Oct., 1999) Vol. 28, No. 4-5, pp. 284. print.
Meeting Info.: 16th Annual Symposium on Nonhuman Primate Models for AIDS Atlanta, Georgia, USA October 7-10, 1998
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Thank you,
Zac Lucas

## ABSTRACT #28

### SIV DELTA NEF AND SIV-IL2 ARE PATHOGENIC IN RHESUS MACAQUES

Gart Sawai, Tesi Low, Sabry Hamza, Mike Stout, Michael Ye, Jo Weber, Kim Schmidt, Karen Shaw, and Paul Luciw. Department of Medical Pathology, University of California, Davis, CA

Nef is a multifunctional protein that is important for cellular signaling and intracellular protein trafficking. In SIV-infected rhesus macaques, Nef is critical for induction of high virus load and development of simian AIDS (SAIDS). Viruses containing large deletions in *nef* replicate to low levels and do not cause disease. Moreover, these viruses have shown efficacy as live-attenuated vaccines in adult monkeys. We constructed SIVΔnef, which contains a mutation in the ATG *nef* start codon and deletes 49 amino acids from a region highly conserved among HIV-1, HIV-2, and SIV. One of two macaques inoculated with SIVΔnef showed high virus load and signs of SAIDS at 2 years post-infection (CD4 T-cell depletion, widespread lymphoid abnormalities). Remarkably, immunoblot analysis revealed that this virus produced a truncated Nef protein (tNef). Sequence analysis of virus recovered from this animal at necropsy confirmed that the start codon for Nef was restored and the deleted amino acids were still missing. In a another study, we inoculated eight macaques with SIV-IL2, which inserts the interleukin-2 (IL2) gene into the deleted *nef* region of SIVΔnef. Four of these macaques developed SAIDS. Interestingly, viruses recovered at necropsy from this group of SIV-IL2 infected animals also produced truncated Nef proteins. Sequencing revealed that the Nef start codon was restored and that the most of the IL2 insert was deleted. In addition, time course analysis demonstrated that reversions restoring tNef correlated with increases in virus load. Such reversions, restoring tNef, were not detected in macaques remaining healthy with low virus loads after infection with either SIVΔnef or SIVΔnef/IL2.

Conclusions: There is strong selection pressure *in vivo* to restore the open reading frame for tNef in juvenile macaques infected with SIVΔNef and SIV-IL2. Further analysis will determine whether tNef and/or an alteration in another viral gene are required for SAIDS. Such studies will define which domains of the multifunctional Nef protein are required for high virus load and disease progression *in vivo*. Additionally, these findings have implications on the safety of live-attenuated viral vaccines constructed by deleting viral accessory genes.

## ABSTRACT #29

### GASTROINTESTINAL TRACT AS A MAJOR PORTAL OF VIRAL ENTRY FOLLOWING ORAL INOCULATION WITH SIVsmmPGm5.3.

Shawn P. O'Neil,[1,2] Francis J. Novembre,[1,2] Juliette deRosayro,[1] Carolyn Suwyn,[1] Daniel C. Anderson,[1] Sherry A. Klumpp,[1] Anne Brodie-Hill,[1] and Harold M. McClure.[1,2] Yerkes Regional Primate Research Center[1] and Emory University School of Medicine,[2] Atlanta, GA.

HIV infection following oral exposure is a major mode of mother-to-child transmission and may also occur following oral-genital contact. We are investigating the mechanism of transmission of SIV across the oropharyngeal and gastrointestinal mucosa. Four pigtailed macaques were inoculated via the oral cavity with a cell-free stock of a macrophage-tropic molecular clone of SIVsmmPGb, designated SIVsmmPGm5.3, in doses which ranged from $2 \times 10^4$ to $2 \times 10^6$ $TCID_{50}$. Virus isolation and PCR confirmed that all four animals became infected. In order to determine the initial portals of viral entry, two animals were sacrificed at 5 days post inoculation (dpi) with $1 \times 10^6$ $TCID_{50}$, and virus loads were compared: (1) along the length of the oropharyngeal and GIT mucosa (by *in situ* hybridization, ISH), and (2) among the lymph nodes which drain the alimentary tract (by ISH, quantitative virus culture, and PCR). Productively infected cells were identified within the oropharyngeal cavity and gastrointestinal tract (GIT) of both macaques, with the greatest number of infected cells located within the proximal duodenum of one animal and the pylorus of the stomach in the second animal. Proviral loads were greatest in the medial retropharyngeal lymph nodes (which drain the oral cavity and pharynx) of both animals, however, suggesting that different mechanisms may be involved in transmission across stratified squamous epithelial surfaces (oropharynx) as opposed to simple columnar epithelial surfaces (GIT). Ongoing studies are directed at determining the phenotype of initial target cells and mechanisms of virus dissemination in adult and neonatal macaques, and should provide valuable information about transmission of HIV/SIV across mucosal surfaces. Supported by NIH grants AI38501 and RR-00165.

## ABSTRACT #30

### INDUCTION OF FAS LIGAND EXPRESSION BY AN ACUTELY LETHAL SIMIAN IMMUNODEFICIENCY VIRUS, $SIV_{smmPGm5}$

Shekema Hodge[1], Francis J. Novembre[2], Linda Whetter[1], Harris A. Gelbard[1], and Stephen Dewhurst[1]. [1]University of Rochester Medical Center, Rochester, New York 14642; [2]Yerkes Regional Primate Research Center and Emory University, Atlanta GA 30322

Human immunodeficiency virus type-1 is the causative agent of AIDS, and infects 40 million people. The progression of HIV infection has been linked to early events in the virus-host interaction, which determine subsequent virus load and disease progression. To examine the initial phase of lentivirus infection in a primate host, we have used the SIV/macaque model for AIDS.

We have studied the acute infection of pigtailed macaques with $SIV_{smmPGm5}$, an SIV variant which induces a severe acute disease syndrome. Enteropathy, immune activation and extensive apoptosis, particularly within gut-associated lymphoid tissue, are characteristic of $SIV_{smmPGm5}$ infection. In the present study, we examined whether the ability of this virus to cause widespread apoptosis might be linked to the upregulation of Fas ligand (CD95L) expression in virally infected cells. *In vitro* studies revealed that expression of the viral Nef protein, in the absence of any other viral gene product, was sufficient to upregulate the transcriptional activity of the CD95L promoter, and also to cause cell surface expression of Fas ligand. This upregulation was NFAT dependent (inhibited by cyclosporin A), and did not occur in cells that expressed a mutated derivative of the viral Nef protein, lacking a previously defined immunoreceptor tyrosine-based activation motif. These findings were corroborated by analysis of tissue sections from virally infected macaques. Immunohistochemical staining revealed that Fas ligand expression was upregulated in the GALT of animals that had been experimentally infected with wild-type $SIV_{smmPGm5}$, but not in animals that were infected with a viral mutant lacking the Nef ITAM. These findings suggest that virally mediated upregulation of Fas ligand expression may contribute to the immunopathogenesis of acute $SIV_{smmPGm5}$ infection.

## ABSTRACT #31

### DISTINCT NUCLEOCYTOPLASMIC TRANSPORT MECHANISMS AND THEIR ROLE FOR HIV/SIV PROPAGATION.

Barbara K. Felber[1], A. S. von Gegerfelt[1], V. Liska[2], R. M. Ruprecht[2], H. M. McClure[3], N. Miller[4], and P. Markham[1]; ABL-Basic Research Program, NCI-FCRDC Frederick[1]; Dana-Farber Cancer Institute, Boston[2]; Emory University, Atlanta[3]; NIAID, Bethesda[4]; Advanced BioScience Laboratories, Kensington[5].

All lentiviruses depend on the posttranscriptional regulation mediated by the viral Rev protein binding to the RRE to express their structural proteins. In contrast, type D retroviruses expression is mediated via a cellular protein, TAP, which interacts with the viral CTE. In the presence of the positive acting factors, the RRE- and CTE-containing mRNAs are efficiently transported to the cytoplasm via distinct nuclear export pathways.

To study the role of Rev in virus propagation, we have generated Rev-independent Nef (-) and Nef(+) clones of HIV and SIV and have demonstrated that the Rev/RRE system can be replaced by the CTE, generating stable viruses with lower replicative capacity and infectivity in primary lymphocytes in vitro, and HIV variants with attenuated phenotype in SCID-hu mouse model. To test the in vivo properties, 3 juvenile and 4 neonate macaques were injected intravenously with a Rev-independent Nef(-)SIVmac239. We found that this virus is attenuated in juvenile and neonate macaques. Importantly, infection by the Rev-independent Nef(-) SIV did not cause disease during one year of follow-up. We further tested a Nef(+) variant of the Rev-independent SIV in 6 juvenile macaques and found that the presence of Nef did not change the severe attenuation of the Rev-independent SIV. Therefore, these data demonstrate that the Rev/RRE regulatory mechanism is required for high levels of virus propagation in vivo, and that this posttranscriptional regulatory control plays an important role in the pathogenicity of the HIV/SIV.

Research sponsored in part by the National Cancer Institute, DHHS, under contract with ABL.

## ABSTRACT #32

### A STRAIN OF RHESUS RHADINOVIRUS (RRV 17577) RELATED TO KAPOSI'S SARCOMA-ASSOCIATED HERPESVIRUS (KSHV) ENCODES A FUNCTIONAL HOMOLOGUE OF CELLULAR INTERLEUKIN-6.

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KSHV is currently thought to be the etiological agent for human Kaposi's sarcoma (KS), the commonest neoplastic complication among HIV-AIDS patients. However, mechanisms underlying the association of KSHV with KS remain undefined due to lack of an accessible natural infection model. Recently, we isolated RRV 17577 from an SIV-infected rhesus macaque that developed a premalignant B cell hyperplasia. RRV 17577 is closely related to KSHV, based on genetic colinearity and possession of several analogous open reading frames (ORF), including a homologue of cellular interleukin-6. Due to the B cell stimulatory effects of IL-6, we investigated whether the IL-6-like gene encoded by RRV 17577 (RvIL-6) could be biologically functional.

We now show that recombinant RvIL-6 expressed either in COS-1 cells, or in Sf9 insect cells can support the growth and survival of the IL-6-dependent B9 cell line. Similarly, GST-RvIL-6 produced in *E. coli* dose-dependently stimulated the proliferation of B9 cells. The GST-RvIL6 signal could be specifically blocked by antibodies to gp130, the transmembrane transducer of signals initiated by IL-6, and by a murine antibody to the low affinity IL-6R, suggesting that RvIL-6 may utilize the classic IL-6 receptor system for signaling. However, although anti-gp130 inhibited both GST-RvIL-6 and hrIL-6 with similar kinetics, inhibition of the GST-RvIL-6 signal by α-IL-6R occurred at a relatively higher concentration compared to its inhibition of hrIL-6. This finding is reminiscent of a previous report of human anti-IL-6R blockade of KSHV vIL-6 function or a human myeloma cell line, indicating that the stimulatory effects of viral IL-6 and cellular IL-6 may be mediated by slightly different structural determinants. Together, these results suggest that RRV 17577 encodes an analogue of KSHV vIL-6, and that these genes may exhibit cell stimulatory functions via a shared mechanism. Thus, in an SIV-immunodepressed background, expression of viral IL-6 may trigger the gp130 pathway and induce the development of a neoplastic cell growth environment that results in the complex pathology associated with RRV 17577 infection.